Dihydrotestosterone activates sexual behavior in adult male hamsters but not in juveniles

Russell D. Romeo, Eric Cook-Wiens, Heather N. Richardson, Cheryl L. Sisk*

Department of Psychology, Neuroscience Program, Michigan State University, East Lansing, MI 48824, USA

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Abstract

The effect of an androgenic metabolite of testosterone, dihydrotestosterone (DHT), on reproductive behavior and brain androgen receptor (AR) immunoreactivity was compared in juvenile and adult male Syrian hamsters. Prepubertal and adult animals were castrated and treated with 0, 500, or 1000 μg of DHT daily for 1 week and then tested for their ability to engage in mating behavior. The 1000-μg dose of DHT activated intromissions in adult but not prepubertal males. Brains were collected immediately after the behavioral test to investigate whether the lack of a behavioral response to DHT prior to puberty is associated with fewer AR-immunoreactive (AR-ir) cells in the forebrain nuclei that mediate male sexual behavior. In four of the five nuclei within the behavioral circuit that were examined, the number of AR-containing cells was similar in prepubertal and adult males treated with 1000 μg of DHT. Only in the anterior medial amygdala (MeA) was there a greater number of AR-ir cells in adults. These data indicate that (1) DHT does not activate components of male reproductive behavior prior to puberty and (2) the lack of behavioral responsiveness to DHT in prepubertal males is most likely not related to an overall reduction in ARs within the forebrain circuit that mediates mating behavior. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

As an animal progresses through puberty it experiences profound changes in reproductive physiology and behavior. For instance, puberty in males is marked by both an increase in testosterone production [13,23] and a greater likelihood to engage in sexual behaviors [9,11,21]. Although the pubertal increases in steroid hormone levels and behavior are temporally coupled, elevated testosterone is not the sole explanation for adult expression of mating behavior. For example, when juvenile male rats [1,5,21], ferrets [20], and hamsters [9] are treated with a dose of testosterone that fully activates sexual behavior in adults the juveniles engage in little or no reproductive behavior. Thus, not only do steroid hormone levels increase during pubertal development, but neural responsiveness to these hormones increases as well. The neural mechanisms that mediate the pubertal increase in responsiveness to steroid hormones remain largely unknown. We are studying pubertal maturation in the male Syrian hamster as a model system for understanding mechanisms of developmental change in neural response to steroids.

Metabolites of testosterone such as estradiol [2,3] and dihydrotestosterone (DHT) [2,15,24] are important mediators of reproductive behavior in the male hamster. These estrogenic and androgenic metabolites of testosterone are formed locally in the brain by the intracellular aromatase and reductase enzymes, respectively, and act via estrogen and androgen receptors (ARs) to facilitate expression of mating behavior. Thus, the lack of behavioral response to testosterone in prepubertal males could arise either from a relative lack of estrogenic action, androgenic action, or both. We have previously shown that prepubertal male hamsters are behaviorally unresponsive to estradiol [16], indicating that cellular responses to activation of the estrogen receptor are different in the prepubertal and adult hamster brain. Whether the androgenic metabolite DHT elicits similar cellular and behavioral effects in pre- and postpubertal males is not known, and this question is the focus of the present study.

* Corresponding author. Tel.: +1-517-355-5253; fax: +1-517-432-2744.
E-mail address: sisk@msu.edu (C.L. Sisk).
The forebrain components of the neural circuit that mediates male mating behavior in the Syrian hamster have been identified and functionally characterized [6,7,12, 26,27,31]. The steroid-sensitive cell groups that comprise the limbic components of this neural circuit express relatively high levels of AR, to which DHT binds [28,29]. DHT increases AR immunoreactivity in this circuit in adult males [29,30]. In the present experiment, we compared mating behavior and AR immunoreactivity in prepubertal and adult castrates treated with DHT to determine whether puberty is associated with changes in neural and behavioral responses to a nonaromatizable androgen.

2. Method

2.1. Subjects and treatment

The male Syrian hamsters (Mesocricetus auratus) used in the present study were bred at Michigan State University (East Lansing, MI). All animals were weaned from their mothers at 21 days of age and singly housed in clear polycarbonate cages (37.5 × 33 × 17 cm) with ad libitum access to food (Teklad Rodent Diet No. 8640, Harlan, Madison, WI) and tap water. The animals were maintained on a 14-h light/10-h dark light–dark schedule (lights off at 1200 h EST) and the temperature was kept at 21 ± 2°C. All animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Michigan State University All-University Committee for Animal Use and Care.

All animals were castrated under methoxyflurane anesthesia at either 21 (prepubertal) or 42 (adult) days of age. Hormone treatment began on the day of castration. Males received a daily injection (at 1000 h) of either 500 or 1000 μg of DHT, or the sesame oil vehicle ([n = 5–6]. On the seventh day of treatment (i.e., at either 28 or 49 days of age), all animals were given a 15-min mating behavior test with a hormone-primed estrous female (see below). Immediately following the behavioral test, animals were weighed, euthanized, and perfused as described below. In hamsters, the pubertal increase in testosterone begins at around 28–30 days of age and adult levels are reached between 40 and 50 days of age [23]. Little or no reproductive behavior occurs in gonad-intact hamsters at 28 days of age, whereas 49-day-old males show the full complement of male reproductive behaviors [9]. Thus, treatment and behavioral tests in the current study occurred either before puberty (i.e., treatment and testing at 21 and 28 days, respectively) or in adulthood (i.e., treatment and testing at 42 and 49 days, respectively).

2.2. Tests for male reproductive behavior

All animals were sexually naïve prior to the behavioral test and were tested 2–5 h after lights out (4–7 h after the seventh injection of DHT). The male was placed in a 10-gal glass aquarium (51 × 26 × 31.5 cm) and allowed to acclimate for 5 min before the introduction of the receptive female. Stimulus females were bilaterally ovariectomized under methoxyflurane anesthesia and subsequently made behaviorally receptive by sequential injections of estradiol benzoate (10 μg in 0.05 ml sesame oil, sc, 48 h prior to testing) and progesterone (250 μg in 0.05 ml sesame oil, sc, 4 h prior to testing).

The behavioral tests were videotaped under dim red light illumination with a Panasonic color video camera (WV 3250). Videotapes were scored to assess the amount of time spent in anogenital investigation (AGI) of the female, and the number of correctly oriented mounts, intromissions, and ejaculations achieved by the male. Specific criteria for these behaviors have been described previously [9]. Videotapes were scored by a single experimenter who was blind to the hormonal condition of the animals.

2.3. Tissue collection

Immediately after the 15-min behavioral test, animals were weighed, administered an overdose of sodium pentobarbital (130 mg/kg, ip), and perfused. Prior to perfusion, seminal vesicles were removed, seminal fluid was expressed, and the wet weight was recorded. We were unable to obtain the seminal vesicles from two animals. Animals were intracardially perfused with 100 ml of buffered saline rinse followed by 150 ml of 4% paraformaldehyde. Brains were then removed and post-fixed for 1 h in 4% paraformaldehyde and then transferred to a phosphate-buffered saline (PBS) solution containing 20% sucrose. Approximately 48 h after the brains had been immersed in the sucrose solution, 40-μm coronal sections were made on a cryostat and stored in cryoprotectant at −20°C until the AR immunocytochemistry was performed.

2.4. AR immunocytochemistry

Every fourth brain section from each animal was processed in a single immunocytochemical run. Sections were rinsed five times in 0.1 M PBS to remove the cryoprotectant. Sections were then incubated sequentially in 0.1 M glycine in 0.1 M PBS (30 min), 0.3% H2O2 in PBS (10 min), 4% normal goat serum (Vectastain ABC Kit, Burlingame, CA) in 0.3% Triton X-100 in PBS (PBS–TX, 1 h), 0.25 μg/ml rabbit anti-AR in PBS–TX (PG-21-18a, obtained from G.S. Prins, Michael Reese Hospital, Chicago, IL, 48 h), secondary antibody (goat anti-rabbit, Vectastain Elite Kit, 1:200 in PBS–TX, 24 h), and avidin–biotin–horseradish peroxidase complex (Vectastain ABC Elite Kit, 1:50 in PBS–TX, 2 h). For the chromogen reaction, sections were incubated for 5 min in 0.05% dianaminobenzidine (DAB), 2% 500 nM...
NiCl₂, and 0.075% H₂O₂. Sections were rinsed three times in PBS between incubations in each reagent. All incubations were at room temperature except for the one in primary antibody, which was at 4°C. Sections were mounted on gelatin-coated slides, dried, dehydrated, cleared in xylenes, and coverslipped. To test for non-specific binding, sections were processed as described above, but in the absence of either primary or secondary antibody. Absence of either antibody eliminated all detectable immunoreactivity.

2.5. Analysis of AR areal density

The areal density (cells per unit area) of AR-immunoreactive cells, referred to as the number of AR-ir cells, was determined for the medial preoptic nucleus (MPN), the magnocellular region of the MPN (MPNmag), the posteromedial subdivision of the bed nucleus of the stria terminalis (BNSTpm), the anterior subdivision of the medial amygdala (MeA), and the posterior subdivision of the medial amygdala (MeP). These areas were chosen for analysis because they are the AR-containing forebrain nuclei of the neural circuit that mediates mating behavior [28,31]. For the MPN, MeA, and MeP, bilateral counts were made in two anatomic ally matched sections separated by 160 μm. For the BNSTpm and MPNmag, a bilateral count was made in a single section anatomic ally matched across animals. Using bright-field microscopy, nuclei were centered in the field of view under a ×10 objective with ×10 eyepieces, and then the magnification was increased using a ×40 objective. AR-ir cell profiles that fell within the area of a square ocular grid (62,500 μm²) were counted. Data are expressed as the mean number of AR-ir cells/62,500 μm². Slides were coded so that the experimenter was blind to the age and treatment of the animal during microscopic analysis.

2.6. Statistical analysis

All peripheral, behavioral, and central variables were analyzed by two-way ANOVAs (Age × Dose). Fisher’s PLSD tests and Tukey HSD tests were used to probe significant main effects and interactions, respectively. Differences were considered significant when P < .05. Data are presented as means ± S.E.M.
Fig. 1B; $F(1,28) = 40.78$, $P < .05$. Fig. 1C depicts the significant interaction between age and DHT treatment on the number of intromissions during the behavioral test [$F(2,28) = 3.55$, $P < .05$]. The 1000-mg dose of DHT activated intromissions in adult but not prepubertal males. There was a significant main effect of age on the number of ejaculations such that adult animals engaged in a significantly greater number of ejaculations than the prepubertal animals regardless of the dose of DHT received [Fig. 1D; $F(1,28) = 4.94$, $P < .05$]. None of the prepubertal animals treated with DHT were observed to exhibit any mounting, intromissive, or ejaculatory behavior.

3.2. AR immunoreactivity

Fig. 2 shows the number of AR-ir cells within the brain regions examined. Two-way ANOVAs revealed a significant main effect of DHT treatment on the number of AR-ir cells in the MPN [$F(2,28) = 13.26$, $P < .05$], BNSTpm [$F(2,28) = 12.11$, $P < .05$], and MeP [$F(2,28) = 59.56$, $P < .05$]. Fisher’s PLSD post hoc tests showed that both age groups treated with either the 500- or 1000-mg dose of DHT had a significantly greater number of AR-ir cells than vehicle-treated animals ($P < .05$). ANOVAs revealed significant interactions between age and DHT treatment in both the MPNmag and MeA [$F(2,28) = 3.80$ and $F(2,28) = 3.58$, respectively, both $P < .05$]. Specifically, 500 μg of DHT increased the number of MPNmag AR-ir cells to a greater extent in prepubertal males than in adults. In contrast, 1000 μg of DHT increased MeA AR-ir cell number to a greater extent in adult compared to prepubertal males.

4. Discussion

These data demonstrate that prepubertal males are less responsive than adults to the activational effects of DHT on male reproductive behavior. Therefore, the lack of a behavioral response to testosterone in prepubertal males appears to arise from a lack of responsiveness to both androgenic (present study) and estrogenic action [16]. This experiment further shows that the inability of DHT to activate mating behavior in juvenile males is not associated with a relative lack of DHT-induced ARs in most of the nuclei that comprise the neural circuit mediating male mating behavior. We previously observed a similar dissociation between sexual behavior and brain AR in which juvenile and adult males were treated with testosterone [9]. Testosterone increased the expression of AR in the steroid-sensitive mating circuit in prepubertal males, but did not activate sexual behavior. Thus, the present data are in agreement with this earlier study, and indicate that the presence of ARs in this neural circuit may be necessary, but not sufficient, to facilitate the display of male mating behavior.

Adult males treated with 1000 μg of DHT did have a significantly higher number of AR-ir cells in the MeA compared to the prepubertal males treated with the same amount of DHT. Since 1000 μg of DHT was capable of activating intromissive behavior in the adult but not juvenile males, the greater number of AR-containing cells in the adult MeA is correlated with the adult’s greater responsiveness to DHT on this behavioral measure. However, intracerebral implantation of estradiol, but not DHT, in the medial amygdala of castrated adult males activates their mating behavior, suggesting that androgenic stimulation of this area alone is not sufficient to elicit a behavioral response [25]. Furthermore, testosterone treatment induces equivalent amounts of AR-ir in the MeA (and elsewhere) in gonadectomized prepubertal and adult males, yet prepubertal males still do not engage in mating behavior [9]. Therefore, the greater number of AR-containing cells in the MeA of adult males treated with 1000 μg of DHT is probably not responsible for their greater behavioral responsiveness to DHT. However, the greater number of ARs in the MeA of adult males could mediate neural responsiveness to other motivated behaviors that are influenced by androgens, such as aggression.

It should be noted that the prepubertal males treated with the 500-μg dose of DHT had a greater number of AR-containing cells in the MPNmag compared to the adults.
that were treated with 500 μg of DHT. The functional significance of the higher levels of AR expression in the MPNmag of androgen-treated prepubertal males is unknown. However, this finding further demonstrates the dissociation between AR levels and sexual behavior since these relatively high levels of AR in the prepubertal MPNmag at this dose did not contribute to any behavioral activation in response to DHT.

Although AR protein is present in the behaviorally relevant brain areas of the DHT-treated prepubertal males, it is possible that the ARs are not functional. The ARs do appear to bind DHT, since treating prepubertal males with testosterone or DHT causes translocation of the ARs to the nucleus of the cell (Ref. [9], present data), as indexed by the profound increase in staining intensity in the nuclear compartment of the cell after hormone treatment. Although these ARs apparently bind DHT, they may not activate the appropriate pattern of gene transcription prior to puberty. However, we showed previously that aromatase activity is increased in both prepubertal and adult male hamsters treated with testosterone [18]. Since testosterone-stimulated increases in aromatase activity are via an AR-dependent mechanism in rats [19], this finding suggests that brain AR is functional in prepubertal male hamsters. Thus, a cellular process downstream from AR induction and activation may be immature prior to puberty, preventing the expression of an androgen-dependent behavior.

Expression of adult reproductive behaviors may depend on steroid-independent maturational events that must occur in conjunction with the pubertal rise in testosterone. Thus, prepubertal males may not engage in mating behaviors even when treated with steroids because a steroid-independent neural process or a change in metabolic signals that is permissive for behavior has not yet occurred. However, rats castrated prior to puberty and then treated with hormones in adulthood respond behaviorally to steroids as if they were prepubertal [5], suggesting steroid-independent maturational events are not the only determinant of the pubertal increase in mating behavior.

Alternatively, the difference in behavioral response to DHT in adults and prepubertal males may be related to differences in their hormonal histories. In the present study, prepubertal males experienced extremely low levels of gonadal steroids prior to treatment, whereas adults had experienced 2–3 weeks of increasing testosterone prior to treatment. Male reproductive behaviors are more readily evoked in gonadectomized adults when steroid treatment is begun sooner, rather than later, after castration [4,10,14]. This difference in responsiveness to hormones is presumably because the proteins, neural connections, and other conditions necessary for mating behavior need only be maintained when steroids are replaced at the time of gonadectomy, whereas they need to be restored when steroids are replaced long after gonadectomy. However, “restoration” versus “maintenance” is unlikely to explain the complete absence of reproductive behaviors in DHT-treated prepubertal males compared to adults, because even adult males that have not experienced gonadal hormones for several weeks still show some behaviors after 7–14 days of steroid replacement [4,8,14,22].

Unlike mounting, intromissions, and ejaculations, AGI was activated by DHT in prepubertal males, although to a lesser degree than in adults. The pheromonal stimulation received by the male during chemoinvestigatory behavior is necessary for the subsequent display of mating behavior in this species [27,32]. These results suggest that the pheromonal cues received by a DHT-treated male may be, like the presence of AR, necessary, but not sufficient, to activate the full suite of reproductive behaviors in juveniles. In the chemosensory components of the mating circuit, Fos immunoreactivity, an index of neuronal activation, is increased in a similar fashion in prepubertal and adult males by exposure to female pheromones. However, prepubertal males do not show a pheromone-induced increase in testosterone secretion as the adults do [17]. Therefore, chemosensory information appears to be processed differently before and after puberty, resulting in different physiological responses. Thus, the inability of DHT-treated juveniles to engage in mating behavior may be the result of a lack of integration of chemosensory and steroidal information in the prepubertal brain, which is normally required for the expression of other components of reproductive behavior in males.

DHT was effective in increasing the amount of brain AR-ir and the wet weight of the androgen-responsive seminal vesicles at both ages, indicating that the prepubertal male is responsive to DHT at some level. Importantly, the similar increase in brain AR-ir in the prepubertal and adult males (except in the MeA), suggests that animals exposed to the same dose of DHT received similar central androgenic stimulation, regardless of age. Thus, the inability of peripherally administered DHT to activate mating behavior prior to puberty cannot be fully explained by unequal exposure of the brain to DHT at the two ages.

In sum, these results demonstrate that prepubertal males are less responsive than adults to the behavior-activating effects of DHT. Together with data from a previous study, it appears that the inability of testosterone to activate reproductive behavior in prepubertal males is due to a lack of responsiveness to both androgenic (present study) and estrogenic action [16]. Furthermore, this experiment showed that the inability of DHT to activate mating behavior in juvenile males is not associated with a relative lack of DHT-induced ARs in most of the nuclei that comprise the neural circuit mediating male mating behavior. Thus, the expression of reproductive behavior appears to require not only
androgenic stimulation, but also developmentally timed neural maturation.

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References